

containing the D1 domain, thus demonstrating seroconversion in these patients and detection of antibodies to the CARDS toxin, either as a recombinant protein or as the D1 domain. In the latter assay, the color intensity of each band appears to increase in the samples in a manner consistent with the time course of collection from the patient during the course of the disease (i.e., 1-1<1-2<1-3) (~~Figure 3~~Figures 3A-B).

Map
8/10/09
On page 53, lines ¹⁴16-28, please amend the paragraph as follows.

ELISAs were also carried out on the samples collected from patients 1 and 2 described above (i.e., samples 1-1, 1-2, 1-3, 2-1, 2-2, and 2-3). In these assays, washing at each stage was performed at least three times with PBS and sera and antibodies were diluted in 1% BSA in PBS. Each well of ~~Immulon~~IMMULON 4 HBX Immunoplates (Dynox) was coated overnight at 4°C with 50 µl of rCARDS toxin/D1 (1 µg/well) diluted in carbonate/bicarbonate buffer (32 mM Na₂CO₃, 64 mM NaHCO₃). Individual plates were washed, 100 µl of 1 mg/ml (wt/vol) BSA in PBS was added to each well, and incubation continued for two hours at room temperature. After washing, 50 µl of diluted human serum samples (1/50 to 1/3200) were added to each well, and plates were incubated for two hours at room temperature. Then, plates were washed, and 50 µl of diluted (1:1000) alkaline phosphatase (AP)-conjugated goat-antihuman IgG (Zymed) were added to each well. Plates were incubated for 1.5 hours at room temperature, washed and 50 µl of substrate solution [p-nitrophenyl phosphate (PNPP)/0.1M Tris pH 9.6] was added and plates were incubated at room temperature for 30-60 minutes. Absorbance values at 450 nm were determined for each well.

On page 54, lines 14-22, please amend the paragraph as follows.

Additional studies were conducted wherein each well of an ~~Immulon~~IMMULON 4 HBX Immunoplate (Dynox) was coated overnight at 4°C with 50 µl of rCARDS toxin (1, 2 or 3 µg/well) diluted in carbonate/bicarbonate buffer. After washing, 50 µl of diluted human serum